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PRINCIPAL INVESTIGATOR: Timothy J. O'Leary, M.D., Ph.D.
Jeffrey D. Seidman, M.D.

CONTRACTING ORGANIZATION: Armed Forces Institute of Pathology
Washington, DC 20306-6000

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Seidman, Jeffrey D., M.D.

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Factors influencing the prognosis of women with surgically treated breast cancer were investigated using patients whose cancers were examined at the AFIP between 1970 and 1980. During this time, effective adjuvant therapy was not available to treat early breast cancer. Social security numbers for a subset of these women were obtained by crossmatching AFIP records with the DEERS database; deaths were determined by matching the National Death Index and the Equifax National Death Search. Histologic sections were graded for nuclear pleomorphism, tubule formation, and mitotic index. Lymph node status and tumor size were determined. Estrogen receptor (ER) status was determined immunocytochemically. Proliferation rate and ploidy were determined using flow cytometry. Women who survived at least three years following initial diagnosis had a survival rate influenced by the number of "positive" lymph nodes, histologic tumor grade, and ER status. Although in the short term, ER (+) have a more favorable prognosis than those who do not, in the long term this finding is reversed, with ER(+) tumors having 20 year survival of 20% lower than those with ER(-) tumors. This factor is independent of node status, patient age, tumor size, histologic grade, ploidy or proliferation rate.

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Timothy Foley 20 March 1997

PI - Signature Date

TABLE OF CONTENTS

INTRODUCTION

Breast cancer is one of the most common causes of morbidity and mortality in women. It's treatment is complicated by a natural history characterized by late recurrences (often 20 years or more after an apparently successful surgical treatment), and a lack of reliable methods by which to assess the prognosis of any individual patient. Even lymph node status, the most reliable indicator of disease dissemination, is of marginal utility in treatment decision making. Up to 30% of women with node-negative disease will go on to die from metastatic cancer, while at least 30% of women with positive lymph nodes may be successfully treated using surgery alone.

One results of the lack of accurate prognostic information is adjuvant treatment which is, for many patients, clearly unnecessary. At this point, most node-negative patients with tumors greater than about 1.0 cm will receive chemotherapeutic intervention. Retrospective natural history information strongly suggests that for most of these patients, this treatment is an unnecessary source of expense and morbidity. Unfortunately, no reliable way to determine which patients will develop disseminated metastases and die without this adjuvant treatment exists.

The objective of this study was to determine if analysis of military personnel and dependents treated for breast cancer between 1970 and 1980 would provide insight into this problem. During this era, there was no effective adjuvant chemotherapy for breast cancer in widespread use. Similarly, antiestrogens, such as Tamoxifen, were utilized only for the treatment of widespread disease. Thus information which would enable reliable prediction of long-term survival in this patient cohort would be expected to provide information on patients who could be expected to survive, long-term, without the use of adjuvant chemotherapy.

BODY

Methods and Materials

Inclusion Criteria:

One thousand and twenty-four patients representing service members and military dependents treated in Armed Forces Medical Treatment facilities, and whose biopsy and/or mastectomy specimens were examined at the Armed Forces Institute of Pathology between 1970 and 1980, were included in the study. Only patients for whom social security numbers were provided, and whose records indicated that tissue blocks had been submitted to the AFIP were included. This represented approximately 8% of the breast cancer patients whose tissue blocks were submitted to AFIP during this ten year interval.

Follow-up:

During this period, medical records for service menbers were maintained under the sponsor's social security number, which was generally available in the AFIP record. The patient name and sponsor's SSN were matched against the Defense Enrollment and Eligibility System (DEERS) database, and the patient SSN thus obtained.

The patient name and SSN were submitted for National Death Index (NDI) Searches and for searches using the Equifax National Death Search (ENDS). The patient name and demographics returned by these searches was matched against those originally submitted to validate the death information. Patients for which SSN data were determined to be invalid, or for which a death certificate was returned with a name or demographics incompatible with the medical record, were deleted from the study.

The ENDS database reliably identifies approximately 80% of deaths, and the NDI database holds information on 98-99% of all deaths since its implementation in 1978. Properly used, the specificity of identification is nearly 100% [1].

Patients whose names and SSN's matched either of these databases were considered dead. When discrepancies existed between dates of death shown in the two databases, the information from the NDI was used.

Patients for whom no match could be found in either database were considered to be alive as of the follow-up date. Errors of classification would be expected to result in understatement of the significance of any potential prognostic factor.

Clinical Information:

Patient age and the date of initial surgery was obtained from the AFIP database. The lymph node status was determined from the original surgical pathology reports when reported numerically, or was otherwise determined, when possible, by examination of slides on file at the AFIP. Similarly, tumor size was determined from the surgical pathology report; estimates of size were performed for some small tumors based upon presentation on the histologic slides. A histologic grade was assigned by a pathologist, using the Elston modification of the "Bloom Richardson and Scarff" grading system. This grading system takes into account the degree of differentiation of the tumor as measured by the amount of tubule formation and the degree of nuclear pleomorphism, and the proliferative activity of the tumor using the mitotic index. The result is a score ranging from 3 to 9.

Flow Cytometry

Flow cytometric examination was performed using nuclei disaggregated from the paraffin blocks using the "Hedley Method." Aneuploidy and S-phase fraction were determined using the

"Multicycle" program.

Estrogen Receptor Status

Estrogen receptor status was determined immunocytochemically when possible, using standard methods. Scoring was performed using the semiquantitative "H-score" method. An "H-score" of 50 was used as a cutoff for determining estrogen receptor positively.

Statistical Analysis

Statistical analysis was performed using the *Statistica* program package. Correlations were assessed using contingency table analysis or regression methods, as appropriate. Survival analysis was performed using Kaplan-Meier plots. Univariate effects for categorical variables were assessed using the log-rank (Cox-Mantel) statistic or using the life table regression (model). Multivariate analysis was performed by using the life-table regression method with "backward" elimination of variables.

When information for a particular case was not available for analysis, the case was excluded from the univariate analysis. Multivariate analyses were carried out using both "case exclusion" methods and regression to impute the missing variable. In all cases, both analyses yielded similar results. In this report, significance values are given for the analysis using imputation.

Results

Six hundred ninety cases were available for analysis, based on the availability of tissue sufficient to perform flow cytometric examination, together with apparently valid clinical follow-up. Examination of raw follow-up data revealed that the survival statistics were not representative of

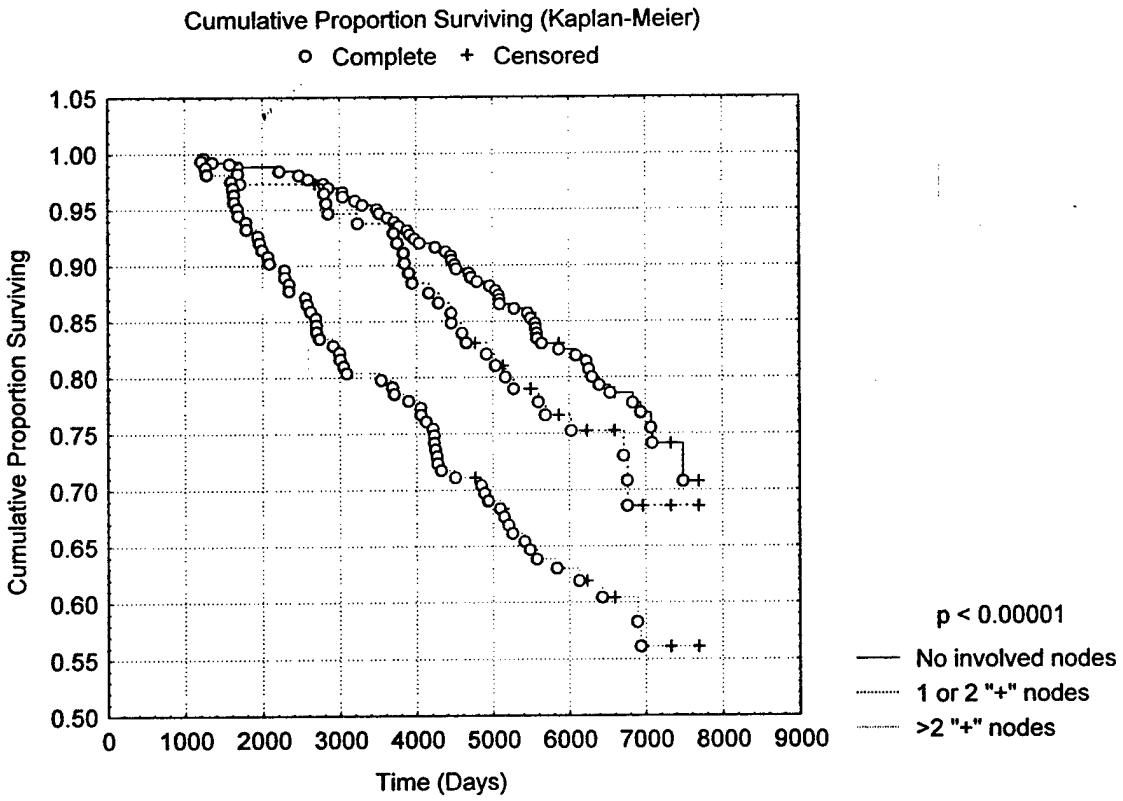
the population at large, in that many fewer early deaths were reported than would be expected. Analysis suggested that the reason for this was that the DEERS system was being implemented during the study period. As a result, women who did not survive for a significant length of time were never enrolled in DEERS, and were thus not available to the study. In order to eliminate the effects of this statistical bias, the study aims were revised, so that emphasis would be placed on long-term survival of women who were alive three years after diagnosis. All women with survival times shorter than three years were excluded from further analysis. Comparison of raw survival curves for this group with those in the literature show comparability. Because the goal of the original study was to determine the effects of various prognostic factors on survival over the long term, this redesign is compatible with the original study objectives.

Factors with Independent Prognostic Value

The following factors were found, in multivariate analysis, to have independent value in predicting the long-term outcome of women with breast cancer.

1. Lymph node status and number of lymph nodes involved. Women with involvement of three or more lymph nodes at the time of mastectomy had a significantly poorer long-term prognosis than women with involvement of fewer nodes. While the adverse effect of lymph node involvement appeared to increase with every involved node, the differences in survival between women with no nodes and either 1 or 2 involved nodes were small (figure 1).

Figure 1. Survival as a Function of lymph Node Status

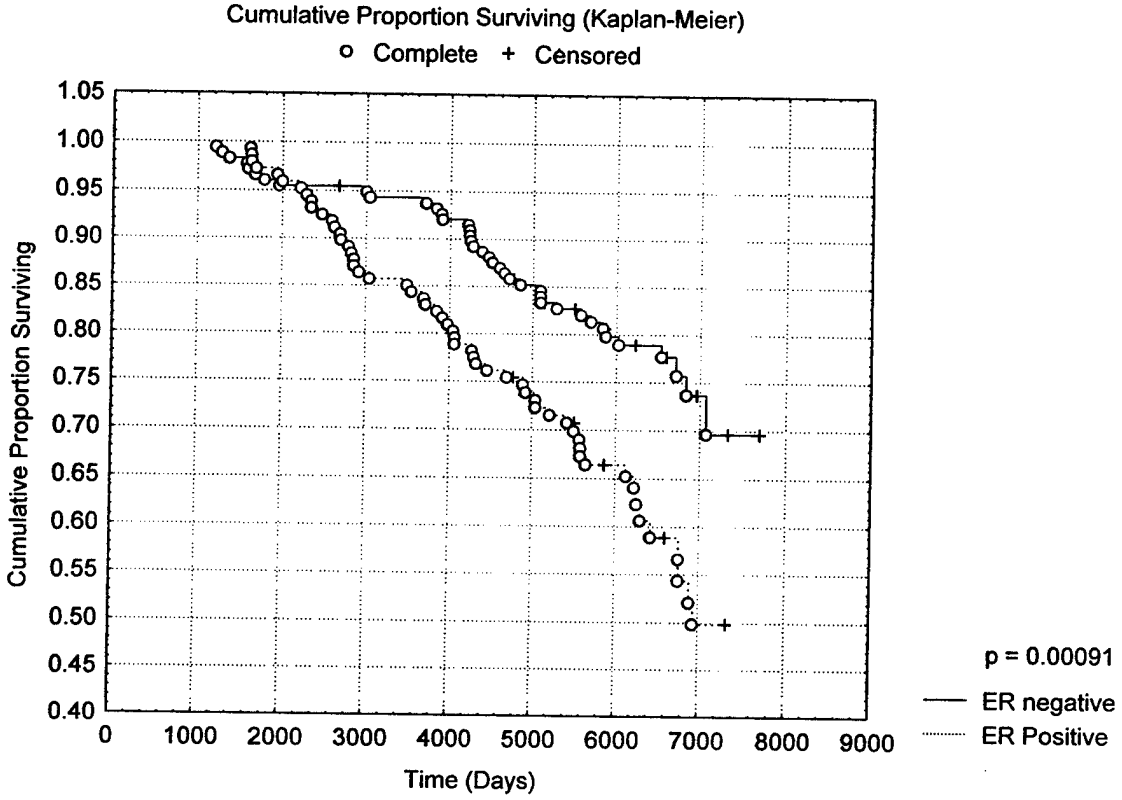


The association between lymph node status and survival is well known, and recent studies [2] have demonstrated that the risk of recurrence for women increases with number of involved nodes for at least seven years following initial diagnosis.

2. Estrogen receptor status: Estrogen receptor status was a strong predictor of outcome (figure 2). In the short term (less than three years), patients with estrogen receptor positive tumors have a better prognosis than those whose tumors do not exhibit estrogen receptors. For women who have survived for three or more years, however, the reverse effect is noted and the prognosis for long-term survival is actually worse for ER-positive tumors. This apparently paradoxical result has been previously observed [3-5]; in this study we have

demonstrated, we believe for the first time, that this effect is independent of other "classical" prognostic factors, including lymph node status, tumor size, histologic grade, proliferative rate of the tumor, and patient age.

Figure 2: Survival as Function of Estrogen Receptor (ER) Status



3. Histologic grade: The histologic grade of the tumor demonstrated marginally significant independent prognostic significance in this study. Noteworthy, however, is the fact that this effect can be attributed almost entirely to the effect of very well differentiated tumors (Elston score 3) on the outcome. Among more poorly differentiated tumors, an effect of histologic grade on outcome cannot be discerned from this data set.

The coefficients and significance levels for the life table regression model are shown in Table 1.

Table 1: Life Table Regression Model

Dependent Variable: Survival times in days

$Chi^2 = 29.7771$ df = 3 p = .00000

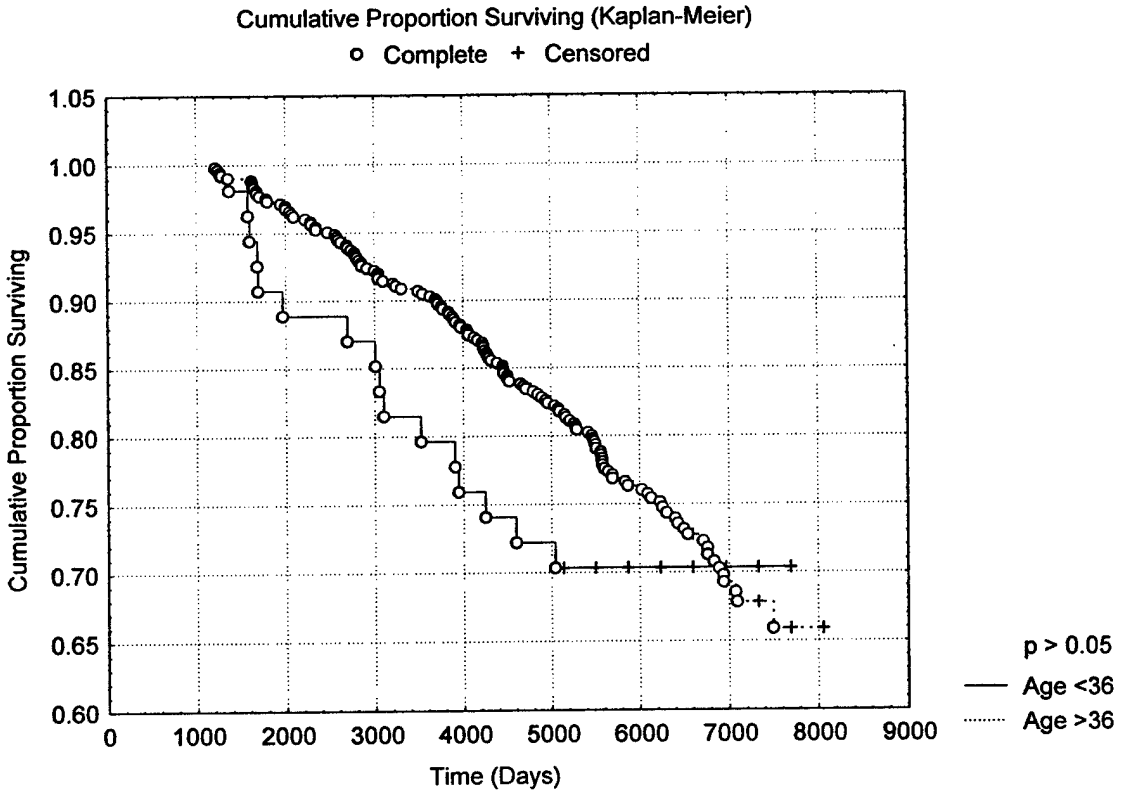
	Beta	Standard Error	t-value	exponent beta	Wald Statist.	p
POSITIVE	.057668	.012272	4.699201	1.059364	22.08249	.000003
TOTAL SC	.036578	.016758	2.182772	1.037255	4.76449	.029060
HGROUP	.393295	.107754	3.649935	1.481855	13.32202	.000263

Factors Without Demonstrable Independent Prognostic Value

1. S-phase fraction and mitotic activity index: These factors were strongly correlated with each other, but did not predict survival. Survival curves for patients with S-phase fractions above and below the median for the study group were virtually identical, as were those for patients with mitotic activity indices above and below the median. Inclusion of this variable in multivariate analysis did not suggest an interaction with other variables.

2. Patient age: Overall, there was no demonstrable effect of patient age on survival, whether age was considered as a continuous variable in a life table regression model, as a dichotomous variable (above and below age 50 - typically chosen as an age division for pre- and postmenopausal), or as a dichotomous variable for patients above and below age 35. Examination of survival curves for patients younger and older than age 35 (figure 3) suggests a poorer prognosis for the younger patients, in conformance with the literature results for overall survival. The effect does not reach statistical significance however, perhaps as a result of the relatively small number of uncensored observations in the young age group.

Figure 3. Survival as a Function of Age



3. Tumor size: Tumor size was not significantly associated with prognosis in the long term.

Discussion

The results outlined above are, for the most part, similar to that which may be inferred from earlier papers. Other work has suggested, but not demonstrated conclusively, that S-phase fraction and tumor size are associated with adverse prognosis in the first three years after diagnosis, but that the effect after that is minimal. Similarly, the long-term effect of lymph node status has been well established.

The major new result from the work, to date, is the unequivocal demonstration that estrogen

receptor - positive tumors have a worse prognosis than do estrogen receptor - negative tumors for women who have survived three or more years following initial diagnosis, and that this effect is independent of histologic grade, tumor size, lymph node status, proliferation rate or patient age. The reason for this paradoxical effect is as yet unknown, and awaits further study.

CONCLUSIONS

Lymph node status, tumor size, estrogen receptor status and histologic grade are independent prognostic factors for long-term survival in patients who have survived three or more years after an initial diagnosis of invasive breast cancer. The effect of estrogen receptor status is opposite that seen in studies of short-term survival however, complicating its use as a tool for helping to decide on initial treatment, and making it unlikely that this variable should be included in any panel used for determining whether adjuvant chemotherapy is warranted in a given case. Although it seems likely that the current practice of avoiding chemotherapy in patients with small, well differentiated, node-negative tumors is warranted, regardless of the estrogen receptor status of that patient, the definition of larger patient groups not requiring adjuvant therapy will require the use of prognostic factors more powerful than those examined in this report. As such prognostic factors are examined on this patient group, supplemental reports will be filed.

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